DP Barcode: D351036 MRID No.: 474180-03

DATA EVALUATION RECORD
HONEY BEE - ACUTE CONTACT & ORAL LC_{50}TEST

i 141-1

1. CHEMICAL: Fosetyl-AL PC Code No.: 123301

2. TEST MATERIAL: Fosetyl-AL Purity: 986 g/kg (98.6%)

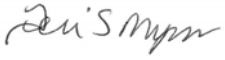
3. CITATION

Authors:	Schmitzer, S. and N. Stork
Title:	Laboratory Testing for Toxicity on the Acute Contact and Oral Toxicity of Fosetyl-AL to Honey Bees (*Apis mellifera* L.) (Hymenoptera, Apidae)
Study Completion Date:	September 24, 1999
Laboratory:	Institut für Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany
Sponsor:	Rhône-Poulenc Agro, Ecotoxicology Department, Sophia Antipolis, France
Laboratory Report ID:	6300036
MRID No.:	474180-03
DP Barcode:	D351036

4. REVIEWED BY: John Marton, Staff Scientist, Cambridge Environmental, Inc.

Signature:  **Date:** 06/04/08

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental, Inc.

Signature:  **Date:** 06/16/08

5. APPROVED BY: Ron Dean, {Specialty}, OPP/EFED/ERB-{Section}

Signature: **Date:**

6. DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to honey bees via oral and contact exposure routes. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the

conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS:

Scientific Name of Test Organism:	*Apis mellifera* L.
Age of Test Organism at Test Initiation:	4-6 week old females
Type of Concentrations:	Nominal (Contact) and Actual Uptake (Oral)
Definitive Test Duration:	48 hours (contact) and 96 hours (oral)

8. CONCLUSIONS:

The honey bee, *Apis mellifera*, was exposed to Fosetyl-AL for 48 and 96 hours in the contact and oral tests, respectively. The contact and oral nominal concentrations were 0 (untreated and solvent controls), 410, 512, 640, 800 and 1000 µg ai/bee. The actual intake concentrations of Fosetyl-AL in the oral toxicity test were 487.9, 570.9, 712.5, 882.7 and 1128.3 µg ai/bee. By 96 hours in the oral test, mortality was 0% in the control and 46.7, 76.7, 70.0, 86.7 and 93.3% in the 487.9, 570.9, 712.5, 882.7 and 1128.3 µg ai/bee treatment groups, respectively. At test termination, all surviving bees appeared healthy and normal. By 48 hours in the contact test, mortality was 3.3% in the 640 µg ai/bee treatment groups, and 0% in the controls and remaining treatment groups. No abnormal behaviors were noted. **The LC_{50} value for the oral test was 496 µg ai/bee. The LD_{50} value for the contact test was >1000 µg ai/bee. As a result, EXP10369F (Fosetyl-AL) is categorized as practically non toxic to honey bees *on an acute contact basis*.** The NOAELs for the oral and contact tests were <488 and 1000 µg ai/bee, respectively.

This study is scientifically *sound/unsound* and *satisfies/does not satisfy* the EFED concerning the guideline requirements for a contact toxicity test with honey bees

(Subdivision L, ï 141-1 or 850.3020). **This study is classified as *ACCEPTABLE/SUPPLEMENTAL/INVALID*.**

Results - Oral Test:

LC_{50}: 496 µg ai/bee	95% C.I.: 356-529 µg ai/bee
NOAEL: <488µg ai/bee	Probit Slope: N/A
LOAEL: 488 µg ai/bee	

Results - Contact Test:

LD_{50}: >1000 µg ai/bee 95% C.I.: N/A
NOAEL: 1000 µg ai/bee Probit Slope: N/A
LOAEL: >1000 µg ai/bee

9. ADEQUACY OF THE STUDY:

A. Classification: Acceptable/Supplemental/Invalid

B. Rationale:

C. Repairability:

10. GUIDELINE DEVIATIONS: This study was conducted following guidelines outlined in EPPO 1992, Guideline on test methods for evaluating the side-effects of plant protection products on honey bees, Bulletin OEPP/EPPO Bulletin 22, 203-215 1992, No 170. There are no OPPTS guidelines for honey bee acute oral toxicity testing, therefore, the following deviations are noted with respect to OPPTS 850.3020, Honey Bee, Acute Contact Toxicity Test:

1. OPPTS guidance recommends that the relative humidity be maintained between 50 and 80%; however, humidity ranged from 42 and 62%.
2. Each bee in the contact test received two 5 µL droplets of the test material in the solvent, while OPPTS guidance states that the volume applied to each bee should not exceed 5 µL.

These deviations do/do not impact the acceptability of the study.

11. SUBMISSION PURPOSE: This study was submitted to provide the acute effects on Honey Bees (*Apis mellifera*) following oral and contact exposure to Fosetyl-AL.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: Species of concern (*Apis mellifera, Megachile rotundata,* or *Nomia melanderi*)	*Apis mellifera*
Age at beginning of test:	4-6 week old females
Supplier:	On-site honey bee colonies

Guideline Criteria	Reported Information
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	Test chambers were stainless steel cages measuring 10 x 8.5 x 5.5 cm. The front of the cage was a removable glass sheet, the bottom was perforated with 98 ventilation holes (ø 1 mm), and the inner walls were lined with filter paper.
Lighting:	Constant darkness except during observations.
Temperature:	28-29°C
Relative humidity:	42-62%

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	No range-finding data were provided.
Reference toxicant test?	Perfekthion EC (Dimethoate; 395.7 g/L). Honey bees in the toxic standard control group were treated with 0.2 µg ai/bee for both tests.
Method of administration:	Oral test: 22-25 mg of Fosetyl-AL contaminated honey were offered in syringes; duration of uptake did not exceed 3 hours Contact test: two single 5 µL droplets of Fosetyl-AL in solvent was planted on the ventral thorax of each bee using a Burkard-Applicator
Nominal doses:	Oral test: 410, 512, 640, 800 and 1000 µg ai/bee Contact test: 410, 512, 640, 800 and 1000 µg ai/bee
Controls: Negative control and/or diluent/solvent control	Oral test: Pure honey Contact test: Negative (untreated) and Solvent (Adhäsit)
Number of colonies per group:	Oral test: 3 reps per treatment group, with 10 bees per rep Contact test: 3 reps per treatment group, with 10 bees per rep
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	Oral test: N/A- no solvent was used Contact test: Adhäsit. The solvent was used to improve the adhesion of the droplet on the bee body. The solvent is non-toxic to honey bees.

Guideline Criteria	Reported Information
Feeding:	Oral and Contact Tests: Commercial honey *ad libitum*; was given directly after treatments in syringes. No replacements of the syringes were necessary during the 48-96 hour exposure periods.
Observations period:	Oral test: 1, 2, 4, 24, 48, 72 and 96 hours- due to increasing mortality at test termination, the study was extended to 96 hours Contact test: 1, 2, 4, 24 and 48 hours

13. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM (98) 17; and Chemikaliengesetz (Chemicals Act) der Bundesrepublik Deutschland (ChemG), Anhang 1 (Annex 1), 2002.
Control performance:	Oral test: 0% in the negative control and 93.3% in the toxic standard group Contact test: 0% in both controls and 96.7% in the toxic standard group
Raw data included:	Yes
Signs of toxicity (if any) were described?	Yes

Mortality - Oral Test

Dosage µg ai/bee (actual intake)	No. of bees	Percent Mortality (%)	
		Hour of Study	
		48	96
Test Substance			
Negative Control	30	0	0
488	30	13.3	46.7
571	30	46.7	76.7
713	30	33.3	70.0
883	30	66.7	86.7
1128	30	90.0	93.3
Toxic Standard			
0.2 µg ai/bee	30	93.3	93.3

Observations:

By 96 hours in the oral test, mortality was 0% in the control and 46.7, 76.7, 70.0, 86.7 and 93.3% in the 487.9, 570.9, 712.5, 882.7 and 1128.3 µg ai/bee treatment groups, respectively. From 1 to 72 hours, bees were noted exhibiting moving coordination problems and apathy. At test termination, all surviving bees appeared healthy and normal. The study authors reported an LD_{50} (with 95% C.I.) value of 461.8 (367.6-580.2) µg ai/bee.

By test termination, mortality was 93.3% in the toxic standard group. After fours hours, bees in the toxic standard group were observed with coordination problems, apathy and nervous behavior.

Mortality - Contact Test

Dosage µg ai/bee	No. of bees	Percent Mortality (%)	
		Hour of Study	
		24	48
Test Substance			
Untreated Control	30	0	0
Solvent Control	30	0	0
410	30	0	0
512	30	0	0
640	30	3.3	3.3
800	30	0	0
1000	30	0	0
Toxic Standard			
0.2 µg ai/bee	30	96.7	96.7

Observations:
By 48 hours in the contact test, mortality was 3.3% in the 640 µg ai/bee treatment groups, and 0% in the controls and remaining treatment groups. No abnormal behaviors were noted. The resulting LD_{50} value was >1000 µg ai/bee.

By test termination, mortality was 96.7% in the toxic standard group.

Statistical method: The oral LD50 of the test substance was estimated with Probit Analysis (according to Finney 1971). The computer program used to perform the statistical analyses was EASY ASSAY Critical Values (Ratte, 1997).

Reported Statistical Results - Oral Test:

LC_{50}: 461.8 µg ai/bee
95% C.I.: 367.6-580.2 µg ai/bee
NOAEL: Not Reported
Probit Slope: N/A
LOAEL: Not Reported

Reported Statistical Results - Contact Test:

LD_{50}: >1000 µg ai/bee 95% C.I.: N/A
NOAEL: 1000 µg ai/bee Probit Slope: N/A
LOAEL: >1000 µg ai/bee

14. VERIFICATION OF STATISTICAL RESULTS:

Statistical method: Mortality did not exceed 3.3% in the contact test. Because the mortality was within the maximum allowable control mortality per OPPTS Guidance (≤10%), the reviewer visually determined the toxicity values for the contact test. The oral LD_{50} (and 95% C.I.) was determined using the moving average method via Toxanal statistical software. Typically, Fisher's Exact test is used to determine NOAEL values for mortality, however, the number of organisms exceeded 20 at each level, making this test unusable. Therefore, the reviewer analyzed replicate % survival using the non-parametric Kruskal-Wallis test via Toxstat statistical software because the data did not meet the assumptions of ANOVA. Normality was tested using the Chi-square and Shapiro-Wilks tests and the homogeneity of variance was tested using the Hartley and Bartlett's tests. The results for the oral test were based on actual uptake concentrations and the results for the contact test were based on the nominal concentrations.

Results - Oral Test:

LC_{50}: 496 µg ai/bee 95% C.I.: 356-529 µg ai/bee
NOAEL: <488µg ai/bee Probit Slope: N/A
LOAEL: 488 µg ai/bee

Results - Contact Test:

LD_{50}: >1000 µg ai/bee 95% C.I.: N/A
NOAEL: 1000 µg ai/bee Probit Slope: N/A
LOAEL: >1000 µg ai/bee

15. REVIEWER↘S COMMENTS:

The reviewer's results for the contact test were identical to those of the study authors; however, the reviewer's LC_{50} result for the oral test was associated with a narrower 95% confidence interval. Therefore, the reviewer's results are reported in the Conclusions section of this DER.

The reviewer's non-parametric analysis of the mortality only detected a significant difference at the highest treatment level relative to the control. However, the reviewer felt that the ≥14% mortality at all treatment levels was biologically significant. Therefore, the reviewer visually determined the NOAEL value to be less than the lowest treatment level.

The in-life portion of the definitive oral and contact tests was conducted between May 31 and June 4, 1999.

16. REFERENCES:

Barrett, K.L., Grandy, N., Harrison, E.G., Hassan, S.A. and Oomen, P. 1994: SETAC- Guidance document on regulatory testing procedures for pesticides with non-target arthropods. 28-30 March 1994, IAC Wageningen, The Netherlands.

Chemikaliengesetz der Bundesrepublik Deutschland (ChemG), Anhang 1 in der Fassung der Bekanntmachung vom 25. Juli 1994 (BGBI. I S. 1703) mit Anderungen vom 27. September 1994 (BGBI I S. 2705) und 14 Mali 1997 (BGBI I S. 1060).

EC Agrochemical Registration Directive (DS65) (Directive 91/414/EEC). ANNEX II. Requirements for the dossier to be submitted for the authorization of a plant protection product.

EPPO 1992: Guideline on test methods for evaluating the side-effects of plant protection products on honey bees, Bulletin OEPP/EPPO Bulletin 22, 203-215 1992, No. 170.

Finney D.J. 1971: Probit Analysis. 3rd Edition, Cambridge University Press, London.

OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997 [C(97)186/Final], Environment Directorate, Organization for Economic Cooperation and Development, Paris 1998.

Ratte H.T. 1995: EASY ASSAY, Critical Values, © SPiRiT, Hans Toni Ratte, Aachen 1997.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
1128.3	30	28	93.33334	4.339964E-05
882.7	30	26	86.66666	2.973807E-03
712.5	30	21	70	2.138698
570.9	30	23	76.66666	.261144
487.9	30	14	46.66667	42.77678

THE BINOMIAL TEST SHOWS THAT 0 AND 570.9 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 496.1209

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.6829165	496.1209	356.191	529.4212

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
2	.2465814	1	.3264233

SLOPE = 3.841497
95 PERCENT CONFIDENCE LIMITS = 1.933926 AND 5.749069

LC50 = 461.787
95 PERCENT CONFIDENCE LIMITS = 306.6181 AND 546.7314

LC10 = 215.6957
95 PERCENT CONFIDENCE LIMITS = 69.67348 AND 318.9363

% Survival, 96 hours; ug ai/bee
File: 8003ps Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.206	4.356	6.876	4.356	1.206
OBSERVED	0	7	6	5	0

Calculated Chi-Square goodness of fit test statistic = 4.2237
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

% Survival, 96 hours; ug ai/bee
File: 8003ps Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 1866.667

W = 0.963

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Critical W (P = 0.05) (n = 18) = 0.897
Critical W (P = 0.01) (n = 18) = 0.858
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Data PASS normality test at P=0.01 level. Continue analysis.



% Survival, 96 hours; ug ai/bee
File: 8003ps       Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance
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These two tests can not be performed because at least one group has
zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

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% Survival, 96 hours; ug ai/bee
File: 8003ps        Transform: NO TRANSFORMATION

              KRUSKAL-WALLIS ANOVA BY RANKS  -  TABLE 1 OF 2
--------------------------------------------------------------------------------
                              TRANSFORMED   MEAN CALCULATED IN      RANK
GROUP    IDENTIFICATION          MEAN         ORIGINAL UNITS         SUM
----- --------------------    ----------   ------------------  -----------
  1            neg control     100.000            100.000          51.000
  2                  487.9      53.333             53.333          39.500
  3                  570.9      23.333             23.333          23.500
  4                  712.5      30.000             30.000          30.000
  5                  882.7      13.333             13.333          16.500
  6                 1128.3       6.667              6.667          10.500
--------------------------------------------------------------------------------

  Calculated H Value =  13.519         Critical H Value Table =  11.070
  Since  Calc H > Crit H  REJECT Ho:All groups are equal.



% Survival, 96 hours; ug ai/bee
File: 8003ps        Transform: NO TRANSFORMATION

 DUNNS MULTIPLE COMPARISON -  KRUSKAL-WALLIS  -  TABLE 2 OF 2
--------------------------------------------------------------------------------
                                                      GROUP
                      TRANSFORMED  ORIGINAL   0 0 0 0 0 0
GROUP  IDENTIFICATION    MEAN        MEAN     6 5 3 4 2 1
----- --------------- ----------  ---------  - - - - - -
  6            1128.3       6.667      6.667  \
  5             882.7      13.333     13.333  . \
  3             570.9      23.333     23.333  . . \
  4             712.5      30.000     30.000  . . . \
  2             487.9      53.333     53.333  . . . . \
  1       neg control     100.000    100.000  * . . . . \
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* = significant difference (p=0.05)            . = no significant difference
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Table q value (0.05,6) =   2.936              SE =    4.295
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